

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:

Cheng Shen et al.

Examiner:

Anthony J. Weier

Serial No.: 10/825,528

Art Unit:

1761

Filed: April 15, 2004

Confirmation No.:

8389

**FOR ACID BEVERAGE COMPOSITION AND PROCESS FOR MAKING  
SAME UTILIZING AN AQUEOUS PROTEIN COMPONENT****DECLARATION UNDER 37 C.F.R. §1.131**

Dear Sir:

We, Cheng **Shen**, Theresa **Cox**, Daniel W. **Brown**, and Jonathan W. **Delcamp** do hereby declare as follows:

1. We are the inventors of the subject matter disclosed and claimed in U.S. Patent Application Serial No. 10/825,528 (the present application).
2. We understand that the Examiner has rejected claims 1, 5, 6, and 10-16 of the present application as being anticipated under 35 USC §102(e) by U.S. Patent No. 6,811,804 (Patel et al.), which was filed on May 28, 2002 (hereinafter the "Patel critical date"). The present application was filed on April 15, 2004, which is within a 12-month period of the Shen critical date.
3. The subject matter of claims 1, 5, 6, and 10-16 of the present application was conceived and reduced to practice by us before the Shen critical date. To demonstrate this fact, attached hereto as Exhibit 1 is a copy of an 8-page report authored by Cheng Shen titled, "Evaluation of Liquid Isolated Soy Protein in Acidic Beverage Application." The dates have been redacted from this report.
4. On page 4, in Table 1 of Exhibit 1, are four Protein IDs directed to Component (C) of the present application that correspond to Examples 2-5 of said application. Also in Table 1 of Exhibit 1 are four Notebook #s directed to the inventive composition of the present application that correspond to Examples 6-9 of said application.

5. On page 5, in Table 3 of Exhibit 1 are one-month sediment data of the four Notebook #s directed to the inventive composition of the present application. The results of this sediment data appear in Table 1 of the present application in the one month data column for Examples 6-9.
6. On page 5, in Table 5 of Exhibit 1 are four-month sediment data of the four Notebook #s directed to the inventive composition of the present application. The results of this sediment data appear in Table 1 of the present application in the four month data column for Examples 6-9.
7. On page 5, in Table 6 of Exhibit 1 are six-month sediment data of the four Notebook #s directed to the inventive composition of the present application. The results of this sediment data appear in Table 1 of the present application in the six month data column for Examples 6-9.
8. The experimental data of Exhibit 1 was performed prior to the Shen critical date.

We hereby declare that all statements made herein of our own knowledge are true and that all statements based on information and belief are believed to be true and further that these statements have been made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both under section 1001 of Title 18 of the United States Code and that such false statements may jeopardize the validity of the application or any patent issuing thereon.

  
Cheng Shen

07/10/07  
Date

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Theresa Cox

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## Document Profile

## Security

## Revision History

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## Project Card -

**Experimental Report**

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Project Leader: Richard Shen/PROTEIN

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Experimental Title: Evaluation of Liquid Isolated Soy Protein in Acidic Beverage Application

Laboratory Notebook #: E100407-1

Send Final Notification to: Brigid Kappel/PROTEIN, Dan W. Brown/PROTEIN, James L. Holbrook/PROTEIN, Paul V. Paulsen/PROTEIN, Phil S. Kerr/PROTEIN, Richard Shen/PROTEIN, William G. Soucie/PROTEIN, Xiaolin L. Huang/PROTEIN

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# I. Introduction:

The solubility of protein has long been considered as a major factor that cause sediment in acidic beverage. To develop a highly soluble protein at low pH is essentially critical to ultimately eliminate sediment in acidic beverage. All isolate protein we used had high heat treatment and phase transfer (liquid to solids) during spray drying process. Did the high heat treatment damage protein solubility and cause more sediment? Since Dan Brown and Theresa Eggleston just developed a new version of HO 255 proteins and plan to spray dry, it is a good chance to get liquid protein for acid beverage research. The liquid protein was obtained right before spray dry after high heat treatment. This study is to evaluate possibility of liquid protein application in hot filled acid beverage.

# II. Objective:

Determine whether isolated soy protein without high heat spray dry performs better in hot filled acid beverage.

# III. Management Summary:

The liquid proteins (E100332-68-5 modified version and SP 675 , FXP HO220) were evaluated in the 6.25g model. All protein drinks had very small particles (about 1 micron) and higher viscosity. Their suspension stability was excellent over entire storage period in both storage conditions (4 °C and 25 °C). Supro Plus 675 protein (E100332-94-3b) had almost no sediment developed. Other protein drinks had less than 2.5% sediment. Two experiment proteins had excellent stability through out whole storage period at both temperature settings. Supro Plus 675 had very good stability up to four months. Even though FXP HO220 had worst sediment in test proteins, it still gave satisfied low sediment value at 3.3% at room temperature. Smaller particles and higher solubility of liquid protein may make pectin/protein interaction more effectively, which is reflected by better stability. The liquid protein could be a better source for acidic beverage applications.

# IV. Experimental Approach:

## Liquid Protein Evaluation

### 1) Formula

Ingredients	as is %	10000g batch
Liquid protein slurry *	23.6	2360.0
Sugar	10.0	1000.0
Apple juice concentrate	2.1	210.0
2% pectin YM-100L	27.5	2750.0
Phosphoric acid 85%	0.27	27.0
Citric acid	0.27	27.0
Water	36.26	3626.0
<b>Total</b>	<b>100.0</b>	<b>10000.0</b>

\* Set solids at 13.5% in Capro version ISP, then the amount of liquid protein needed to make 6.25g protein per 8 oz serving is  $2.55/80\%/13.5\% = 23.6.0\%$ . For sample E100332-94-1b, it is  $2.55/90\%/13.5\% = 21.0\%$

### 2) Process control

- make 2% pectin slurry at ambient water for 5 min., then heat to 170F for 10 min, then cool to room temperature

- b. add pectin slurry into liquid protein slurry, mix 5 min
- c. add sugar, juice, and acids (No phosphoric acid added in samples E100332-94-1b and 2b)
- d. adjust pH at 3.8 - 4.0
- e. Pasteurize at 190 F for 30 sec.
- f. Homogenize at 2500/500 psi
- g. packaging immediately, hold for 2 min, then cool in ice water

V. **Evaluation:**

Chemical and physical test

1. Solids, Vacuum Oven, IM000340
2. pH, Liquid, IM000366
3. Viscosity Brookfield, 4C and 25 C IM000456
4. Color, Hunter L, a, b, IM000028
5. Particle Size: Malvern Particle Size Analyser
6. Shelf Life: 4°C and 25°C  
(two samples for each setting, and check at 1, 7 day & every month)
7. Transmittance

VI. **Samples/Products Used:**

E100332-94-1b New Protein, Provided by Theresa Eggleston  
E100332-94-2b Capro version of 94-1b, Provided by Theresa Eggleston  
E100332-94-3b Supro Plus 675 TE pilot plant sample, Provided by Theresa Eggleston  
E100332-94-5b FXP HO 220 TE pilot plant sample, Provided by Theresa Eggleston

VII. **Samples/Products Produced:**

E100407-1-1  
E100407-1-2  
E100407-1-3  
E100407-1-5

VIII. **Results and Discussion:**

**PHYSICAL PROPERTIES**

1. pH, solids and viscosity

pH was designed to be 3.8 - 4.0. They were all in the range to ensure proper suspension stability. Solids test was used to monitor formulation. It should be around 14% which were in the bulk range. Viscosity had slight difference in FXP HO220 TE sample. It was low compared to other three proteins based beverages. The viscosity at 4C was double the viscosity at 25C for all samples just like all other similar test done before.

2. Transmittance

Transmittance (T%) is developed to predict suspension stability of soy protein (Supro Plus 675 and FXP HO220 type) based acidic beverages. Generally speaking, T% has to be less than 3% to ensure a good suspension, and less than 1% for better suspension. Both Supro Plus 675 and FXP HO220 made beverages had T% less than 1% indicating a better suspension formed. However, two experiments protein based beverages had T% higher than 10%, but still maintain excellent suspension stability. Those results were confirmed by other tests (ER#4, ER#5). Transmittance does not fit into acidic beverages made from E100332-94 type proteins because they are much soluble at lower pH than Supro Plus 675.

## 3. Particle size D(4,3)

The particle size listed in Table 1 is volume weight mean particle size that emphasis on big tails. They were all very small (around 1 micron) compared to 4-5 micron for commercial protein made beverages (EO#4 and 5). Therefore, they should technically give stable suspension over time.

## 4. Color

The biggest color difference is their whiteness (L value). Supro Plus 675 and FXP HO220 gave more creamy and milky white appearance, while two high soluble proteins (E100332-94-1b and 2b) generated more transparent suspensions.

Table 1 Physical Properties

Notebook #	E100407-1-1	E100407-1-2	E100407-1-3	E100407-1-5
Protein ID	E100332-94-1b	E100332-94-2b	SP 675 TE	FXP HO220 TE
pH	3.86	3.96	3.94	3.86
Solids %	13.79	13.98	13.79	13.98
Viscosity at 4 °C cps	20.85	23.25	21.10	16.06
Viscosity at 25 °C cps	10.35	11.75	10.55	8.05
Transmittance %	14.56	14.54	0.98	0.86
Mean Size um	1.01	1.46	0.75	0.77
L	70.34	68.55	78.74	78.08
a	-1.29	-1.36	-0.57	-0.60
b	13.22	13.31	11.47	12.21

## SUSPENSION STABILITY EVALUATION

Suspension stability was monitored at week 1, month 1, 2, 4, and 6. In the first two months, sediment at all temperature settings were monitored, and only 4C and 25C sediment were tested afterwards.

## Week One

There were no visible sediment developed for all samples except at 41C - an accelerated temperature test. Apparently, two test proteins were much better than Supro 675 and FXP HO220.

Table 2 Sediment development after one week storage

Notebook #	E100407-1-1	E100407-1-2	E100407-1-3	E100407-1-5
Protein ID	E100332-94-1b	E100332-94-2b	SP 675 TE	FXP HO220 TE
Sediment at 4 °C	0.00	0.00	0.00	0.00
Sediment at 25 °C	0.00	0.00	0.00	0.00
Sediment at 41 °C	0.50	0.94	2.00	2.59

## Month One

Two experimental proteins based beverages had visible sediment developed while Supro 675 and FXP HO220 based drinks still hold very well at both 4C and 25C temperature conditions. However, their sediment at 41C stated to pick up at much fast pace, especially FXP HO220 which had 5.87% sediment already. It may indicate that this beverage will have lot more sediment in the long run.

Table 3 Sediment development after one month storage

<b>Notebook #</b>	<b>E100407-1-1</b>	<b>E100407-1-2</b>	<b>E100407-1-3</b>	<b>E100407-1-5</b>
<b>Protein ID</b>	E100332-94-1b	E100332-94-2b	SP 675 TE	FXP HO220 TE
<b>Sediment at 4 °C</b>	0.76	0.50	0.00	0.00
<b>Sediment at 25 °C</b>	1.16	0.59	0.00	0.50
<b>Sediment at 41 °C</b>	1.40	1.07	1.45	5.87

## Month Two

The winners at 25C and 4C are Supro Plus 675 and FXP HO220. They are still better than two experiment proteins at month two. Supro Plus 675 sample at 41 C started to have heavy sediment developed (6.76%) while FXP HO220 sample's sediment at 41C kept growing to close to 8%. After two months storage at 41C, it was demonstrated that Supro Plus 675 and FXP HO220 type protein based acidic beverages may develop heavy sediment after longer shelf storage. There was no correlation found between sediment at 41 C and at 4C or 25C, partial reason probably because those four proteins were very different in their functions, ie. solubility.

Table 4 Sediment development after two months storage

<b>Notebook #</b>	<b>E100407-1-1</b>	<b>E100407-1-2</b>	<b>E100407-1-3</b>	<b>E100407-1-5</b>
<b>Protein ID</b>	E100332-94-1b	E100332-94-2b	SP 675 TE	FXP HO220 TE
<b>Sediment at 4 °C</b>	1.25	0.50	0.00	0.50
<b>Sediment at 25 °C</b>	2.19	1.71	0.50	0.50
<b>Sediment at 41 °C</b>	3.05	3.04	6.76	7.95

## Month Four

For the first time, FXP HO220 made beverage had higher sediment (3.28%) than other three proteins. Supro Plus 675 had lowest sediment in all samples at only 1.23%, E100332-94-2b was closely followed at 1.58%. They were all excellent in terms of sediment development over four months storage when compared to commercial samples (ER#5).

Table 5 Sediment development after four months storage

<b>Notebook #</b>	<b>E100407-1-1</b>	<b>E100407-1-2</b>	<b>E100407-1-3</b>	<b>E100407-1-5</b>
<b>Protein ID</b>	E100332-94-1b	E100332-94-2b	SP 675 TE	FXP HO220 TE
<b>Sediment % at 4 °C</b>	1.11	1.11	0	2.16
<b>Sediment % at 25 °C</b>	2.14	1.58	1.23	3.28

## Month Six

Finally, two experiment proteins outperforms Supro Plus 675 and FXP HO220 at month six mark, which verified accelerating test result showing Supro Plus 675 and FXP HO220 had higher sediment even at week one. Supro Plus 675 had less sediment than FXP HO220, but both were double or triple the amount of sediment than two experimental proteins made beverages. E100332-94-2b, a Capro version of E100332-94-1b had lowest sediment in all samples.

Table 6 Sediment development after six months storage

<b>Notebook #</b>	<b>E100407-1-1</b>	<b>E100407-1-2</b>	<b>E100407-1-3</b>	<b>E100407-1-5</b>
<b>Protein ID</b>	E100332-94-1b	E100332-94-2b	SP 675 TE	FXP HO220 TE
<b>Sediment % at 4 °C</b>	2.22	1.10	1.15	5.44
<b>Sediment % at 25 °C</b>	3.16	2.13	4.88	6.56

**SEDIMENT DEVELOPMENT OVER SHELF LIFE**

The sediment development over shelf life was plotted in Figure 1 and 2. At ambient environment, Supro Plus 675 and FXP HO220 based beverages had lower sediment than two experiment protein made beverages, and then took off after two to four months storage with dramatically sediment increase. Two experiment protein based drinks had relatively flat sediment development after one month storage. Their sediment increase was very limited. At refrigeration temperature, the overall sediment was lower than at ambient environment, Supro Plus 675 performs best even after six months storage, while FXP HO220's sediment took off at month four. Two experiment proteins had no difference in their beverages' sediment change. All were with slight increase over six months shelf life.

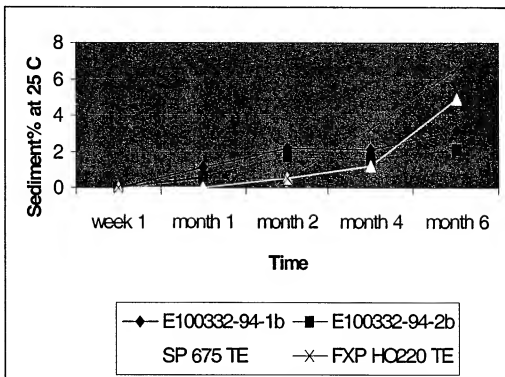


Figure 1 Sediment development over shelf life at 25 C



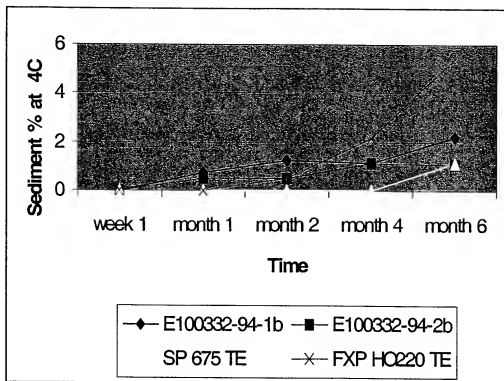


Figure 2 Sediment development over shelf life at 4 C

#### IX. Conclusions:

Liquid soy protein isolate could be applied directly into acidic beverages without spray dry process that gave better stability over long shelf life. Those beverages had smaller particle size, lower transmittance (for Supro Plus 675 and FXP HO220) and stable suspension. Two experiment proteins had excellent stability through out whole storage period at both temperature settings. Supro Plus 675 had very good stability up to four months. Even though FXP HO220 had worst sediment in test proteins, it still gave satisfied low sediment value at 3.3% at room temperature. The liquid protein could be a better source for acidic beverage applications.

#### X. Recommendations:

Further test liquid protein in acidic beverage at different protein levels as well as under high heat treatment.

#### Document Attachments:

Author \_\_\_\_\_

Date \_\_\_\_\_

Project Leader \_\_\_\_\_

Date \_\_\_\_\_

Additional Approver \_\_\_\_\_

Date \_\_\_\_\_

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Additional Approver

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Date